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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/417,386	10/13/1999	JONATHAN M. ROTHBERG	15966-539	7371

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MINTZ LEVIN COHN FERRIS GLOVSKY POPECO
ONE FINANCIAL CENTER
BOSTON, MA 02111

EXAMINER
TAYLOR, JANELL E

ART UNIT	PAPER NUMBER
1655	

DATE MAILED: 01/29/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/417,386	Applicant(s) ROTHBERG ET AL.	
	Examiner Janell Taylor Cleveland	Art Unit 1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 November 2001* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-7,9-20 and 27-32* is/are pending in the application.

4a) Of the above claim(s) *28-30 and 32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-7,9-20 and 27-32* is/are rejected.

7)☐ Claim(s) *1,3-17 and 31* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Continued Prosecution Application

1. The request filed on November 30, 2001 for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) is acceptable and a CPA has been established. An action on the CPA follows.

2. This is a CPA of applicant's earlier application. All claims are drawn to the same invention claimed in the earlier application and could have been finally rejected on the grounds and art of record in the next Office action if they had been entered in the earlier application. Accordingly, **THIS ACTION IS MADE FINAL** even though it is a first action in this case. See MPEP § 706.07(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no, however, event will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Election/Restrictions

1. Newly submitted claims 28-30 and 32 are directed to an invention that is independent or distinct from the invention originally claimed for the following reasons:

2. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1, 3-7, 9-20, 27, and 31, drawn to a method of screening a population of nucleic acids, classified in class 435, subclass 6.

II. Claims 28-30 and 32, drawn to a method of identifying a novel nucleic acid sequence, classified in class 435, subclass 6.

3. Inventions I and II are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different modes of operation, different functions, or different effects (MPEP § 806.04, MPEP § 808.01). In the instant case the different inventions have different modes of operation, each capable of use without the other. Furthermore, the groups contain materially different method steps.

4. Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, claims 28-30 and 32 are withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03.

Claim Objections

5. Claims 1 and 31 are identical. Applicant is required to cancel the claim(s), or amend the claim(s) or rewrite the claim(s) so they are no longer identical.

6. Claims 3-17 are objected to because of the following informalities: the claims depend from rejected claim 2. Appropriate correction is required.

7. Claim 6 is objected to because of the following informalities: the claim recites "DNA molecules" when it is believed that "RNA molecules" was meant, since the claim from which it depends refers to RNA and not DNA. This is believed to be a spelling error. Appropriate correction is required.

Claim Rejections - 35 USC § 112

8. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

9. Claims 7 and 27 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. These claims depend from claim 1, and recite that the partitioning step comprises various embodiments. However, this is confusing because claim 1 already recites that the partitioning step comprises digesting cDNA molecules with one or more restriction enzymes. Therefore, it is confusing as to whether or not the other embodiments of partitioning are in addition to the digesting of the cDNA or if they replace that step. Appropriate correction is required.

Claim Rejections - 35 USC § 102

10. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

11. Claims 1, 31, 19, and 27 are rejected under 35 U.S.C. 102(e) as being anticipated by Austin et al. (USPN 6,132,965).

Claims 1 and 31 are drawn to a method of screening a population of nucleic acids for a novel sequence, the method comprising: providing a population of cDNA molecules derived from a population of RNA molecules, partitioning said population into one or more subpopulations of nucleic acids, wherein partitioning comprises digesting the cDNA molecules with one or more restriction enzymes; identifying a first nucleic acid sequence in the subpopulation of nucleic acid sequences; and comparing the first nucleic acid sequence to a reference nucleic acid sequence or sequences, wherein the absence of the first nucleic acid sequence in the reference nucleic acid or nucleic acid sequences indicates the first nucleic acid is a novel nucleic acid sequence. Claim 19 is drawn to the method of claim 1, wherein comparing is by hybridizing the first nucleic acid sequence to one or more of the reference nucleic acid sequences. Claim 27 is drawn to the method of claim 1, wherein the partitioning comprises one or more processes chosen from the group consisting of: a) isolating nucleic acids from different cell types, b) separating the nucleic acids in the subpopulation by size, c) amplification that provides a subpopulation of nucleic acids, d) preferentially amplifying 5' terminal sequences of the nucleic acids, e) preferentially amplifying interior sequences of the nucleic acids, and f) preferentially amplifying 3' terminal sequences of the nucleic acid.

Austin et al. teaches "RNA prepared by conventional methods from a first cell population and RNA from a second cell population are separately reverse-transcribed and second-strand synthesized to form two pools of double-stranded cDNA (*providing a*

population of cDNA molecules derived from a population of RNA molecules), a tester pool comprising sequences of the mRNA species desired to be enriched for, and a driver pool comprising the sequences desired to be subtracted from the tester pool. The two pools may be fragmented by endonuclease digestion (restriction endonuclease or non-specific endonuclease) if desired to enhance hybridization efficiency. (*partitioning said population into one or more subpopulations of nucleic acids, wherein said partitioning comprises digesting the cDNA molecules with one or more restriction enzymes*). The driver pool and tester pool are denatured and mixed together in a reaction mixture under hybridization conditions and incubated for a suitable hybridization period. The reaction mixture is contacted with a ligand which binds the recoverable label on the driver cDNA and which can be readily recovered from the reaction mixture (e.g., avidin attached to magnetic beads), such that a substantial fraction of the driver cDNA and any tester cDNA hybridized thereto is selectively removed from the reaction mixture. (*identifying and comparing the first nucleic acid sequence to a reference nucleic acid sequence, wherein the absence of the first nucleic acid sequence in the reference nucleic acid indicates the first nucleic acid is a novel nucleic acid sequence*). The enriched (subtracted) tester cDNA pool may be subjected to one or more additional rounds of subtractive hybridization with a pool of labeled driver cDNA..." (Col. 22, lines 40-70).

Therefore, Austin teaches all of the limitations of claims 1, 31, 19, and 27.

Claim Rejections - 35 USC § 103

12. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. Claims 3-7, 18, and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Austin et al.

Claim 3 is drawn to the method of claim 1, further comprising partitioning the RNA molecules. Claims 4-6 are drawn to the cDNA population being derived from the 5' ends, the interior regions, and the 3' ends of the RNA molecules, respectively. Claim 7 is drawn to the partitioning of the molecules comprising hybridization of a probe nucleic acid sequence to the population of nucleic acids. Claim 18 is drawn to the method of claim 1, wherein comparing is by determining at least a portion of the nucleotide sequence of the first nucleic acid sequence and comparing the nucleotide sequence to the nucleotide sequence of one or more reference nucleic acids.

Claim 20 is drawn to a method for equalizing the representation of nucleic acids in a population of nucleic acids, the method comprising in order the steps of: providing a population of cDNA molecules derived from a population of RNA molecules, wherein said population comprises a first nucleic acid and a second nucleic acid having a nucleic acid sequence distinct from the first nucleic acid, and wherein said first nucleic acid is present at a higher level in said population than said second population; partitioning said population into one or more subpopulations of nucleic acids, wherein said partitioning comprises digesting the cDNA molecules with one or more restriction

enzymes; and lowering the level of said first nucleic acid sequence relative to the level of said second nucleic acid sequence in the subpopulation of nucleic acid sequences, thereby equalizing the representation of nucleic acids in said population of nucleic acids.

As disclosed above, Austin et al. teaches "RNA prepared by conventional methods from a first cell population and RNA from a second cell population are separately reverse-transcribed and second-strand synthesized to form two pools of double-stranded cDNA a tester pool comprising sequences of the mRNA species desired to be enriched for, and a driver pool comprising the sequences desired to be subtracted from the tester pool. The two pools may be fragmented by endonuclease digestion (restriction endonuclease or non-specific endonuclease) if desired to enhance hybridization efficiency. The driver pool and tester pool are denatured and mixed together in a reaction mixture under hybridization conditions and incubated for a suitable hybridization period. The reaction mixture is contacted with a ligand which binds the recoverable label on the driver cDNA and which can be readily recovered from the reaction mixture (e.g., avidin attached to magnetic beads), such that a substantial fraction of the driver cDNA and any tester cDNA hybridized thereto is selectively removed from the reaction mixture. The enriched (subtracted) tester cDNA pool may be subjected to one or more additional rounds of subtractive hybridization with a pool of labeled driver cDNA..." (Col. 22, lines 40-70).

Austin et al does not teach partitioning the RNA molecules before creating cDNA, or what portion of the RNA molecule the cDNA portion is derived from, or hybridization

of a probe nucleic acid sequence to the population of nucleic acids, or that the comparing of sequences is by determining the nucleotide sequence, or equalizing the representation of the molecules.

It would have been obvious to one of ordinary skill in the art at the time of the invention that the RNA molecules of interest may have been partitioned prior to cDNA copies being made from it. This would have been obvious because it would have been desirable to separate, or partition, the RNA population from other cellular material, or to partition certain RNA populations from others by using restriction enzymes or hybridization techniques. This would have allowed a certain portion of the cellular RNA to be characterized without the interference of undesired RNA transcripts, or other cellular materials. It would also have been obvious that the cDNA population may have been derived from any portion of the RNA that was desired, whether that be the 5' end, the 3' end, or an interior portion. This is because it would have been desirable to use any of those segments, depending on what the desired portion of the RNA was, and it was well known in the art that copies of any of those portions of RNA were easily copied into cDNA molecules. Also, it would have been obvious to hybridize a probe to the population of nucleic acids. This would have been obvious because it would have been another way of identifying which molecules of the target nucleic acids hybridized with molecules of the reference nucleic acid, instead of using ligands as taught by Austin. Probes would have been obvious because it was well known that they were easily detectable by fluorescent detection methods, and hybridizable to an array, etc. It would have also been obvious to use the method of Austin et al. to equalize the representation

of nucleic acids in a population. This is because Austin taught that two populations were hybridized together, and this would have equalized the representation of the populations by having only sequences which hybridized remain. This would have been obvious because it would have been advantageous to have two equalized population when comparing sequences, for instance.

14. Claims 9-17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Austin et al as applied to the claims above, and further in view of Sytkowski et al. (USPN 6,177,244).

Claim 9 is drawn to the method of claim 1, further comprising ligating adapter oligonucleotides to the termini of the digested cDNA molecules, thereby producing ligation products. Claim 10 is drawn to the method of claim 9, further comprising amplifying the ligation products. Claim 11 is drawn to the method of claim 10, further comprising separating the amplified products. Claim 12 is drawn to the method of claim 11, wherein separating is by gel electrophoresis. Claim 13 is drawn to the method of claim 11, wherein the first nucleic acid sequence is identified by comparing the size of one or more digestion products produced by a member of the subpopulation of nucleic acids to the sizes of fragments generated by the same restriction enzyme or enzymes in said reference nucleic acid or nucleic acids. Claim 14 is drawn to the method of claim 11, further comprising recovering one or more size-separated digestion products; reamplifying the recovered products; and separating the reamplified products. Claim 15 is the method of claim 14, wherein said separating is by gel electrophoresis. Claim 16 is drawn to the method of claim 15, wherein the first

nucleic acid sequence is identified by comparing the size of one or more digestion products produced by a member of the subpopulation of nucleic acids to the sizes of fragments generated by the same restriction enzyme or enzymes in said reference nucleic acid or nucleic acids. Claim 17 is drawn to the method of claim 9, further comprising: inserting the ligated adapter oligonucleotide into a cloning vector to form a vector-insert; transforming the vector-insert into a suitable host; culturing transformed host under conditions allowing for replication of the vector-insert; recovering the vector-insert from said host; and digesting the vector-insert with one or more restriction enzymes, thereby releasing said insert; and comparing the size of the insert to sizes of fragments generated by the same restriction enzyme or enzymes in said reference nucleic acid.

As disclosed above, Austin et al. teaches "RNA prepared by conventional methods from a first cell population and RNA from a second cell population are separately reverse-transcribed and second-strand synthesized to form two pools of double-stranded cDNA a tester pool comprising sequences of the mRNA species desired to be enriched for, and a driver pool comprising the sequences desired to be subtracted from the tester pool. The two pools may be fragmented by endonuclease digestion (restriction endonuclease or non-specific endonuclease) if desired to enhance hybridization efficiency. The driver pool and tester pool are denatured and mixed together in a reaction mixture under hybridization conditions and incubated for a suitable hybridization period. The reaction mixture is contacted with a ligand which binds the recoverable label on the driver cDNA and which can be readily recovered from

the reaction mixture (e.g., avidin attached to magnetic beads), such that a substantial fraction of the driver cDNA and any tester cDNA hybridized thereto is selectively removed from the reaction mixture. The enriched (subtracted) tester cDNA pool may be subjected to one or more additional rounds of subtractive hybridization with a pool of labeled driver cDNA..." (Col. 22, lines 40-70).

Austin et al. does not teach ligating adapter oligonucleotides to the termini of the digested cDNA molecules, amplifying those products, separating the amplified products using gel electrophoresis, comparing the sizes of the populations, recovering the size-separated products and reamplifying them, or inserting the ligated adapter oligonucleotide into a cloning vector to form a vector-insert; transforming the vector-insert into a suitable host; culturing transformed host under conditions allowing for replication of the vector-insert; recovering the vector-insert from said host; and digesting the vector-insert with one or more restriction enzymes, thereby releasing said insert; and comparing the size of the insert to sizes of fragments generated by the same restriction enzyme or enzymes in said reference nucleic acid.

Sytkowski et al teaches a method used to isolate genes expressed differentially between two cell types or between cells treated in two different ways, or for isolation of differences between genomic DNA sequences. In a process of subtractive hybridization, Sytkowski et al. teaches the use of ligating linker, or adapter, oligonucleotides to the ends of the cDNA molecules. Sytkowski also teaches multiple rounds of subtractive hybridization, amplification, and gel electrophoresis. (Col. 48, line 4 through Col. 49,

line 60). Sytkowski et al. also teaches the formation of a subtractive library using a cloning vector, and comparing the size fragments (Col. 50, lines 14-20).

It would have been obvious to one of ordinary skill in the art at the time of the invention to combine the teachings of Austin and Sytkowski. This is because it would have been advantageous to use an adapter which was ligated to the end of the cDNA in order to amplify and identify the desired sequences. Furthermore, it would have been obvious to one of ordinary skill in the art at the time of the invention to use a gel to compare sizes of fragments. This would have allowed one of ordinary skill in the art to distinguish between fragments of different samples. Also, it would have been obvious to repeat those steps of amplification and subtractive hybridization in order to obtain the highest percentage of novel nucleic acids. It would have also been obvious to use a vector in order to create a library that would have been useful at a later date for comparison.

Summary

Claims 28-30 and 32 are subject to the restriction requirement, and are non-elected by original presentation. Claims 1, 3-17, and 31 are objected to. Claims 7 and 27 are rejected under 35 U.S.C. 112, second paragraph. Claims 1, 31, 19, and 27 are rejected under 35 U.S.C. 102(e). Claims 3-7, 9-18, and 20 are rejected under 35 U.S.C. 103(a). No claims are allowable.

Conclusion

15. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Janell Taylor Cleveland, whose telephone number is (703) 305-0273.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached at (703) 308-1152.

Any inquiries of a general nature relating to this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers related to this application may be submitted by facsimile transmission. Papers should be faxed to Group 1634 via the PTO Fax Center using (703) 305-3014 or 305-4227. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG (November 15, 1989.)

Janell Taylor Cleveland

January 25, 2002

W. Gary Jones
Supervisory Patent Examiner
Technology Center 1600